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厦门大学

硕 士 学 位 论 文

酞菁红区荧光探针在生物大分子检测中的应用及其用于生物成像的可行性初探

Red Emitting Phthalocyanines Used as Fluorescent Probes for the Analysis of Biomacromolecules and Premilinary Invesitgation of Their Applications in Bioimaging

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摘 要

由于在长波区域具有吸收或发射特性的天然和人工合成的物质极少，因而在进行生物、生化和临床等复杂试样的测定时，使用长波发射荧光试剂可有效避开背景荧光和散射光的干扰，且光漂白作用小，与传统的荧光试剂相比具有很大优越性。能发荧光的酞菁化合物即是一类红区发射的荧光试剂。本论文的工作围绕酞菁红区荧光探针在生物大分子分析及成像检测中的应用而展开，共分五章。

第一章：首先对红区及近红外荧光探针的应用进展进行了简要介绍。由于酞菁化合物作为红区荧光试剂的研究构成了本文的主要部分，本章还对酞菁化合物的分子结构和光谱特性以及它在生物模拟酶、光动力治疗、生化分析领域的应用做了介绍。

第二章：本章建立了简便、快速测定硫酸软骨素的荧光增强分析法。通过荧光光谱筛选实验发现，具有共轭结构的阳离子头部和长碳链尾部的阳离子表面活性剂可几乎完全猝灭四磺基铝酞菁（Tetrasulfonated Aluminum Phthalocyanine，AlS_4Pc）的荧光。而在带有磺基阴离子的硫酸软骨素(Chondroitin Sulfate，CS)存在下，体系荧光显著恢复。以 AlS_4Pc-阳离子表面活性剂离子缔合物为红区荧光探针，考察了其对 CS 的荧光恢复响应行为，发现离子缔合物的荧光恢复程度与CS浓度存在良好的相关性，据此实现了复杂样品中CS简便、准确的测定。本章工作分为两部分：由于前期工作筛选出的猝灭效果较好的阳离子表面活性剂有两种，由这两种猝灭剂参与的体系的灵敏度和线性范围不同，各具特色，因此对两个体系分别进行了考察，并建立了两种 CS 的荧光增强测定法。

第三章：本章建立了简便、快速测定溶菌酶的荧光增强分析法。带有强阴离子的黏多糖肝素(Heparin，HP)可诱导阳离子铝酞菁[Tetra(trimethyammionio) Aluminum Phthalocynine，TTMAAlPc]聚集而导致荧光显著猝灭。由于溶菌酶对黏多糖具有催化降解作用，因而可水解HP为小分子片段，从而破坏TTMAAlPc-HP的聚集缔合平衡，使TTMAAlPc被释放，体系荧光将因之恢复。结合荧光光谱与荧光各向异性技术对反应机理进行了探讨。据此建立了溶菌酶测定新方法，并实现了复杂样品中的溶菌酶含量的准确测定。

第四章：探讨了红区荧光染料酞菁在指纹成像观测中的应用。考察了几种酞菁染料对潜在指印的成像效果，其中 $Al(SO_2Cl)_4TSP$ 对于玻片上油指印的染色效果最为理想，进一步的对染料溶剂，浓度，染色方法和时间进行了考察和优化，成像结果令人满意。

第五章：为研究红区荧光染料酞菁在细胞中是否有特异性定位，为此首先通过摸索建立了显微注射技术，直接将荧光酞菁染料注射进细胞中，体外培养后观察染料与细胞是否有结合。初步研究的结果表明荧光酞菁化合物具有成为活细胞成像新型荧光探针的潜力。

关键词：酞菁；硫酸软骨素；溶菌酶；含量测定；生物成像

Abstract

Long wavelength emitting fluorescent probes can effectively avoid the interference of background fluorescence and scattered light, as compared with the conventional fluorescent reagents, because the natural and synthetic materials have little absorption or emission in long-wavelength region. Phthalocyanine compounds include one class of fluorescent reagents emitted in red region. This work focus on the applications of red emitting phthalocyanine fluorescent probes on the analysis of biomacromolecules and imaging detection, it is divided into five chapters.

In chapter 1, the developments of fluorescent probes in red and near-infrared region were reviewed, followed by the introduction of phthalocynine compounds about their molecular structure, characteristic of molecular spectra, methods for preparation and the main application areas of these compounds.

In chapter 2, this work aims at developing a novel method for rapid determination of chondroitin sulfate. The fluorescence of tetrasulpnonated aluminum phthalocyanine (AlS_4Pc), a anionic metal phthalocyanine, was quenched dramatically by cationic surfactant in which contains a positively-charged head with a conjugated structure and a long carbon chain as tail through the formation of a almost non-fluorescent association complex. It was found that the ion-association complex (AlS_4Pc-CPB) emitted strong fluorescence in the presence of chondroitin sulfate, due to the ability of chondroitin sulfate to shift the association equilibrium of the ion-association complex that leaded to the release of AlS_4Pc, thus resulting in an increase in the fluorescence of AlS_4Pc. Based on the above-mentioned phenomenon, a novel method for quantitative determination of chondroitin sulfate in practical samples was developed using the ion-association phthalocyanine complex as a fluorescent probe emitting at red-region. Two anionic surfactants quenching the fluorescence of AlS_4Pc with high efficency were screened. It was found that different sensitivity and linear range for the determination of chondroitin sulfate were obtained using the two surfactants as quenchers. Therefore, two determination

systems were investigated separately.

In chapter 3, the main idea of this part of work is to develop a novel method for rapid determination of lysozyme. It was found that the fluorescence of the cationic aluminum phthalocyanine, a red-region fluorescence probe, was extremely quenched in acidic media in the presence of low concentrations of anionic mucopolysaccharide heparin（HP）bearing anionic sulfonic acid groups, due to induced aggregation. The almost non-fluorescent substrate was degraded into small molecule fragments by the hydrolysis of lysozyme, hence, the phthalocyanine molecules aggregated in HP were released, resulted in significant fluorescence recovery of the reaction system. This phenomenon bases the principle of the proposed method. The reaction mechanism was discussed employing fluorescence spectroscopy and steady-state fluorescence anisotropy techniques. Factors which affected the determination were investigated. The established method has been used to the analysis of real samples of lysozyme, the results are in well agreement with those determined by a conventional turbidimetric method.

In chapter 4, preliminary investigation on the the application of fluorescent phthalocyanines for the imaging of potent fingerprints are carried out. Seven fluorescent phthalocyaniens were screened,and factors which affected the detection were investigated. Among phthalocyanine compounds having been investigated, $Al(SO_2Cl)_4TSP$ showed the most effective results for the imaging of potent fingerprints.

In the final chapter, the possibility of fluorescent phthalocyanines for in vivo imaging of cells was prmilinarily investigated. The purpose of this work is to study the location behavior of fluorescent phthalocyanines inside cells. Microinjection techinique was established for injecting fluorescent phthalocyanines into cells directly. Preliminary results of the study showed that fluorescent phthalocyanine compounds have the great potential to be new fluorescent probes for cell imaging in vivo.

Keywords: phthalocyanine;chondroitin sulfate;lysozyme;determination;bio-imaging